First report of leaf spot of spinach (Spinacia oleracea) caused by Myrothecium verrucaria in Italy.

First Report of Leaf Spot of Spinach (*Spinacia oleracea*) Caused by *Myrothecium verrucaria* in Italy

A.Garibaldi, G. Gilardi, S. Franco-Ortega, and M. L. Gullino

Centre of Competence for Innovation in the Agro-Environmental Sector (AGROINNOVA), Largo Paolo Braccini 2, Italy; and M. L. Gullino, AGROINNOVA and DISAFA, Largo Paolo Braccini 2, Grugliasco, Italy.

During the spring of 2015, plants of spinach (*Spinacia oleracea* L.) 'Donkey' were grown using conventional control strategies in plastic houses in the Lumbardy Region near Brescia (Northern Italy). The plants showed symptoms of a previously unknown foliar disease on 15- to 20-day-old plants that consisted of small, circular, sunken, gray-brown spots (1 to 2 mm in diameter) with a well-defined border. As the lesions expanded (up to 30 mm in diameter) they developed concentric rings, coalesced, and then the affected tissues became water-soaked. Creamy to black sporodochia appeared on the affected leaves. Roots were asymptomatic. Five hectares of plastic houses irrigated with well water by overhead sprinklers were affected, with 5 to 10% of plants exhibiting symptoms. Affected plants showed 30 to 60% incidence of diseased leaves. Isolations were carried out on symptomatic leaf-tissue sections (each 1 mm^2) dipped in 1% sodium hypochlorite for 1 min, rinsed in sterilized water, dried on sterilized filter paper, and then placed on potato dextrose agar (PDA) amended with 25 mg/liter of streptomycin sulfate. A white slow-growing fungus was obtained that developed black sporodochia 7 to 10 days after plating, similar to those present on the affected leaves. Conidia were cylindrical, nonseptate, without appendages, measuring 5.8 to 9.2 and 1.9 to 2.8 (avg. of 30 conidia, $7.6 \pm 1.14 \times 2.4 \pm 0.22$) µm. The morphological characteristics of the isolated pathogen corresponded to those of *Myrothecium* spp. (Domsch et al. 1980). The Internal Transcribed Spacer (ITS) region of rDNA was amplified using the primers ITS1/ITS4 and sequenced. BLAST analysis (Altschul et al. 1997) of the 516-bp segment showed a 100% similarity with *M. verrucaria* KR708633.1. A GenBank Accession No. KT354922 was assigned. To confirm pathogenicity, 15-day-old leaf spinach plants cv. Donkey were transplanted in 2-liter pots, filled with a steamed peat, perlite, and sand substrate in a ratio of 60:20:20 vol/vol and maintained in a growth chamber between 20 and 24°C. Five pots per treatment were used with five plants per pot. The artificial inoculation was carried out by spraying leaves with a spore suspension (1×10^5 conidia/ml) prepared from 15-day-old PDA cultures of one representative isolate of the pathogen grown under 12 h of photoperiod at 20°C. Control plants were inoculated with distilled water. Plants were kept covered with plastic bags for 5 days. Spots similar to those of the original plants developed 7 days after the inoculation, with a disease incidence ranging from 60 to 70% of the plants affected. All the noninoculated plants remained asymptomatic. A fungus morphologically identified as *M. verrucaria* was consistently isolated from all the symptomatic plants. The pathogenicity test was conducted twice, showing the same results. This is the first report of *M. verrucaria* on *S. oleracea* in Italy as well as worldwide. The cultivation of spinach for the fresh market is increasing in recent years in northern Italy. Currently, this disease has spread to several farms in the Lumbardy Region, causing limited crop losses

References

Altschul, S. F., et al.. 1997. Nucleic Acids Res. 25:3389. https://doi.org/10.1093/nar/25.17.3389

Domsch, K. H., et al.. 1980. Page 481 in: Compendium of Soil Fungi. Academic Press, New York.